Note

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Title

Determination of MIC Distribution and Epidemiological Cut-Off Values for Bedaquiline and Delamanid in *Mycobacterium tuberculosis* Using MGIT 960/ TB eXiST

Authors

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Abstract

Bedaquiline and delamanid have recently been approved by the regulatory authorities for treatment of multidrug-resistant tuberculosis (MDR-TB). Antimicrobial susceptibility testing is not established for either substance. On the basis of MGIT 960/ TB eXiST we determined a mean bedaquiline MIC of wild-type strains of 0.65 mg/L (median 0.4 mg/L) and an epidemiological cut-off (ECOFF) of 1.6 mg/L; for delamanid a mean wild-type MIC of 0.013 mg/L (median 0.01 mg/L) and an ECOFF of 0.04 mg/L was determined.

Text

Globally, 3.5% of new and 20.5% of previously treated tuberculosis cases were estimated to have been multidrug-resistant (MDR)-TB in 2013 (1). Bedaquiline (Sirturo™; formerly known as TMC207 and R207910, marketed by Janssen Therapeutics, Titusville, NJ, USA) is the lead compound of a series of recently discovered diarylquinolines, first described in 2005 (2). The US Food and Drug Administration (FDA) approved bedaquiline for the treatment of adults with MDR-TB in 2012 (3). Because of the new mechanism of action of bedaquiline – the compound acts via inhibition of mycobacterial ATP synthase (AtpE) – it has been postulated that antimicrobial susceptibility testing (AST) is not needed in patients who have never received bedaquiline (4). However, cross-resistance between bedaquiline and the antimycobacterial drug clofazimine through overproduction of the efflux pump MmpL5 has recently been described (5, 6). Thus, resistance may develop independently of treatment with bedaquiline (2, 7). Delamanid (Deltyba™; previously known as OPC-67683, marketed by Otsuka Novel Products GmbH, Munich, Germany) was approved by the European Medicines Agency (EMA) in April 2014. The mechanism of action of delamanid is incompletely understood; delamanid is suggested to inhibit production of methoxymycolic acid and ketomycolic acid (8). Similar to the related drug PA-824, delamanid is a prodrug requiring activation by the mycobacterial F420 system, including the nitroreductase Ddn (Rv3547) (8-10). Delamanid resistance is thought to arise from mutations in the mycobacterial F420 genes (*ddn*, *fgd1*, *fbiA*, *fbiB*, *fbiC*) associated with the prodrug's activation (8, 11). The spontaneous rate of delamanid resistance has been reported to be as high as 6.44×10^{-6}-4.19×10^{-5}, emphasizing the need to protect delamanid with other active anti-TB drugs during therapy (9).

Initially, AST of bedaquiline was reported using radiometric BACTEC 460TB (BD, Franklin Lakes, NJ, USA), which has since been discontinued (2). Reported minimal inhibitory concentrations (MIC_{90}) for delamanid range from 0.006 mg/L to 0.05 mg/L (depending on the test system) across *M. tuberculosis* isolates (8, 9, 12). Ten years after the drugs' discoveries, established protocols for automated *in vitro* AST of bedaquiline and delamanid are still not available. To establish procedures for bedaquiline and delamanid AST, we used well-characterized, fully drug-susceptible clinical *M. tuberculosis* strains of

bedaquiline and delamanid treatment-naïve patients, MDR-TB strains, and subsequent isolates of a well characterized XDR strain (6). It has been speculated, that the phylogenetic lineage of *Mycobacterium tuberculosis* complex may affect innate drug susceptibility (13). To assess phylogenetic diversity of the set of strains studied all *M. tuberculosis* strains included underwent genotypic characterization by mycobacterial interspersed repetitive units – variable number tandem repeats analysis (MIRU-VNTR) using the GenoScreen MIRU-VNTR Typing Kit (GenoScreen, Paris, France) according to the manufacturer's description. In order to determine the QC (quality control) MIC value, the pan-susceptible *M. tuberculosis* H37Rv reference strain was used. Details about the resistance patterns of the strains and genotypes are shown in Table S1 and S2 (Supplementary Material). With the view to facilitate implementation in the routine laboratories we used the semi-automated MGIT 960 system and the EpiCenter software equipped with TBeXiST module for quantitative drug susceptibility testing (14). The MGIT 960 platform is a fully automated system that uses a fluorescence-quenching-based oxygen sensor for growth detection. This system is widely used in routine laboratories for AST of *M. tuberculosis*. As bedaquiline and delamanid were not available to us as pure substances (supply of bedaquiline was denied, supply of delamanid would have been associated with unacceptable binding conditions) we decided to establish AST using tablet formulations. Based on the accompanying prescription information, the composition and drug content of the tablets was accessible. For bedaquiline the tablet contained 100 mg active compound as well as colloidal anhydrous silica, croscarmellose sodium, hypromellose 2910, lactose monohydrate, magnesium stearate, corn starch, microcrystalline cellulose, and polysorbate 20 (15). For delamanid, one tablet contained 50 mg of active compound and according to the summary of product characteristics as provided by the producer, hypromellose phthalate, povidone, all-rac-α-tocopherol, cellulose, microcrystalline, sodium starch glycolate (type A), carmellose calcium, colloidal hydrated silica, magnesium stearate, lactose monohydrate, hypromellose, macrogol 8000, titanium dioxide, talc, yellow iron oxide (E172) (16). After grinding the tablet, the powder was dissolved in DMSO (Sigma D5879) and stored in small aliquots at - 80 °C. Test concentrations were obtained by serial twofold dilutions in DMSO. After thawing, stock solutions were used for same day experiments. Stability of stock

solutions for both drugs was assessed in parallel. For AST, MGIT tubes supplemented with 0.8 mL of OADC supplement (OADC supplement; Becton Dickinson) were inoculated with 0.2 mL of the drug in DMSO solution and 0.5 ml of the test strain suspension (final DMSO concentration 2.4%). For preparation of the drug-free growth control tube, the organism suspension was diluted 1:100 with sterile saline, and then 0.5 mL was inoculated into the tube (proportion testing) containing 2.4% DMSO (volume/volume). The bacterial suspensions were prepared from MGIT subcultures. Results were interpreted as follows: At the time when the growth unit (GU) of the drug-free control tube was >400, the strain was categorized resistant (R) if the GU of the drug-containing tube was ≥100. If the GU of the drug-containing tube was <100 at this time-point, the strain was categorized as sensitive (S). The MIC of each strain was defined as the lowest drug concentration that was categorized S as per the above definition. According to EUCAST (European Committee on Antimicrobial Susceptibility Testing), the epidemiological cut-off (ECOFF) value is the MIC value identifying the upper limit of the wild-type population (17). The ECOFF can be estimated by visual inspection of a histographic population analysis of the tested strains (eyeball method) or calculated statistically (18, 19). We used visual inspection and a receiver operating characteristic (ROC) curve-based method to determine the ECOFF (20). Drug stability was tested in four series of 11 different drug concentrations in MGIT tubes using *M. tuberculosis* H37Rv as test strain. For the first series, no pre-incubation was chosen. For the second series, MGIT tubes were pre-incubated without bacterial inoculum for one week. For the third series, a pre-incubation time of two weeks was chosen, and for the fourth series a pre-incubation time of three weeks was chosen. The results of all measurements were compared. No difference in susceptibility pattern for all series was detected for bedaquiline. For delamanid, one dilution higher MIC values were measured after two weeks and two dilutions higher MIC values after three weeks indicating a stability issue.

We tested ten wild-type, fully drug-susceptible *M. tuberculosis* strains obtained from bedaquiline and delamanid treatment-naïve patients isolated between 2011 and 2014. Due to the limited amount of bedaquiline and delamanid available, we chose six to ten concentrations for AST (see Tables S1 and S2). For bedaquiline, the arithmetic mean for the wild-type MIC was 0.54 mg/L and the median

was 0.4 mg/L. *M. tuberculosis* H37Rv had a MIC of 0.4 mg/L. In an analysis of 12 MDR and pre-XDR isolates the arithmetic mean of the MIC was 0.77 mg/l, the median of the MIC was 0.8 mg/L (p fully drug-susceptible versus MDR >0.05, non-significant difference between medians). The overall arithmetic mean for the susceptible phenotype was 0.65 mg/L; the overall median was 0.4 mg/L. Two XDR isolates with a bedaquiline-associated resistance mutation (Rv0678 Met1Ala) that also confers cross resistance to clofazimine had a MIC of 6.4 mg/L (6). Using the eyeball method for ECOFF determination, a value of 1.6 mg/L can be supposed (Figure 1A). This eyeball-derived ECOFF was confirmed by a ROC-based method at >90% specificity level. For delamanid, the ten strains from treatment-naïve patients showed MIC values between 0.005 and 0.04 mg/L. The arithmetic mean for the wild-type MIC was 0.016 mg/L; the median was 0.01 mg/L. *M. tuberculosis* H37Rv had a MIC of 0.01 mg/L. The 12 MDR and pre-XDR strains had MIC values between 0.005 and 0.04 mg/L. The overall arithmetic mean for the susceptible phenotype was 0.013 mg/L, the overall median was 0.01 mg/L. 3 XDR isolates of a patient with acquired delamanid resistance (case report in preparation) showed MIC values > 0.32 mg/L (Table S2). We propose an eyeball-ECOFF of 0.04 mg/L (Figure 1B). The ROC curve methodology could not be applied for delamanid, due to the lack of exact MIC values for the three resistotype isolates.

The published MIC values for *M. tuberculosis* H37Rv (bedaquiline MIC 0.03 mg/L, delamanid MIC 0.002 mg/L) are considerably lower than in our study (2, 21). This probably reflects a systematic difference in methodology. Bedaquiline and delamanid both show extensive protein binding, i.e. PK/PD (pharmacokinetics/ pharmacodynamics) data indicate a plasma protein bound fraction > 99.9% (9, 22). It has been shown that the bedaquiline MIC increases in the presence of 5% bovine serum albumin (22). Previous studies determined drug susceptibility mostly in the absence of albumin (21). The albumin content in the MGIT 960 test tube by addition of OADC (this study) or MGIT growth supplement as supplied by BD is approximately 4% (weight/volume), comparable to the physiological plasma protein concentration. A challenge in AST for both substances is their poor solubility in water, a complication known for other antimycobacterial drugs such as ethionamide. Corresponding compounds have to be dissolved in DMSO as solvent and the growth control has to contain the same amount of DMSO to control

for any possible effect on bacterial growth. In general, AST is done using pure substances as provided by the manufacturer. For this study, tablet formulations had to be used, because both producing companies denied the supply of the substances or were unwilling to provide the compound without extensive binding conditions for use and data publication. This is a yet unseen policy for new antimicrobials entering the market, as AST should be established and verified independently by expert laboratories (17, 23). The development and periodic revision of AST guidelines as part of drug development requires close cooperation between academic experts, funding agencies, pharmaceutical companies, and regulatory authorities, as has occurred for antivirals in the past (24).

Our study has several limitations: Most notably, the limited amount of compound available precluded the analysis of a larger strain collection to more precisely determine the ECOFF. The proposed ECOFFs may change slightly with increasing sample size and a finer resolution of drug concentration scaling. In addition, given that bedaquiline and delamanid have only recently entered the market, *M. tuberculosis* isolates with acquired resistance are barely accessible. We established AST (Tables S1 and S2) using a phylogenetically diverse strain set as shown by MIRU-VNTR analysis (Figure S1) in order to measure the variation in the 'wild-type' MIC distribution and to maximize the chance of identifying genotypes that might be intrinsically resistant (13). Further studies evaluating *in vitro* laboratory MIC using pure compound, PK/PD and clinical data from a large number of drug-susceptible and drug-resistant strains are required to define clinical breakpoints (17, 23).

Despite all these limitations our study provides valid AST results. We propose ECOFF values based on population analysis and eyeball-method, which allow discrimination between wild-type and resistotype populations. Our study shows the feasibility of MGIT 960 equipped with TB eXiST for AST of bedaquiline and delamanid in the routine clinical laboratory.

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Transparency declarations

All authors have no conflicts of interest to declare.

Figure

FIG 1 Distribution analysis of MIC values for bedaquiline (A) and delamanid (B). Proposed ECOFF values are marked with an arrow. Wild-type isolates are indicated by grey bars, resistant isolates are indicated by black bars.

Supplementary Data

Table S1 Bedaquiline AST results.

Table S2 Delamanid AST results.

Figure S1 Graphical representation of the relatedness of the strains according to MIRU-VNTR results. The figure contains all strains of the study. The study's XDR strains and one MDR strain share the same MIRU-VNTR pattern.